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Actin and microtubule cytoskeleton interactions

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Plant cytoskeleton consists of two major networks of protein polymers, actin microfilaments (AFs) and microtubules (MTs). These networks perform numerous functions that are essential for cell division and for maintaining the integrity of cytoplasm required for intracellular transport and cell shape. Besides the more or less indirect cooperation between AFs and MTs, their direct interactions through specific physically interacting proteins has been well described in yeast, nematodes, insect and animal cells. Recently, promising candidates for corresponding homologous proteins have been identified in plants, although there is still lack of functional evidence for these interactions. Here we summarize recent advances in our knowledge about the candidate proteins or protein complexes that interact with both AFs and MTs and their role in fundamental cellular and developmental processes.

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Introduction

AFs and MTs, forming two distinct networks, serve as highly dynamic scaffolds for virtually all intracellular processes in eukaryotes. They perform various functions with the assistance of interacting molecular motor proteins, signaling molecules or structure-supporting elements. Although AFs and MTs fulfill many functions independently, they often act in a coordinated manner. The basic question, as put by Yarm *et al.* [1] several years ago, is whether these two networks are coordinated indirectly by independent regulation of one network that subsequently affects the other network, or whether they interact directly through specific bifunctional proteins or multiprotein complexes. In plants, studies based on analyses of cytoskeletal mutants, pharmacological approaches that selectively interfere with AFs or MTs, and microscopical observations have addressed the importance of their mutual coordination in intracellular transport, directional cell expansion and cell division, as reviewed in [2••]. Here we summarize recent progress in molecular biology and modern visualization techniques that provide solid evidence that at least part of the coordination is indeed based on direct physical interactions between AFs and MTs mediated by bifunctional proteins or multiprotein complexes that act as 'matchmakers'. Moreover, some of these interactions are conserved in all eukaryotes including yeast, nematodes, insect, and mammals. For reviews see [1,3,4].

Two of the fundamental cellular processes known to integrate the functions of AFs and MTs in plants are intracellular transport and the formation of the mitotic and cytokinetic apparatus. These processes are essential for the movement of organelles, directional cell expansion ([5], Szymanski, this issue) and also for mitosis [6] and cytokinesis [Van Damme, this issue]. As will be shown here, these processes may indeed involve direct interactions, or 'meetings', between AFs and MTs through specific proteins or protein complexes.

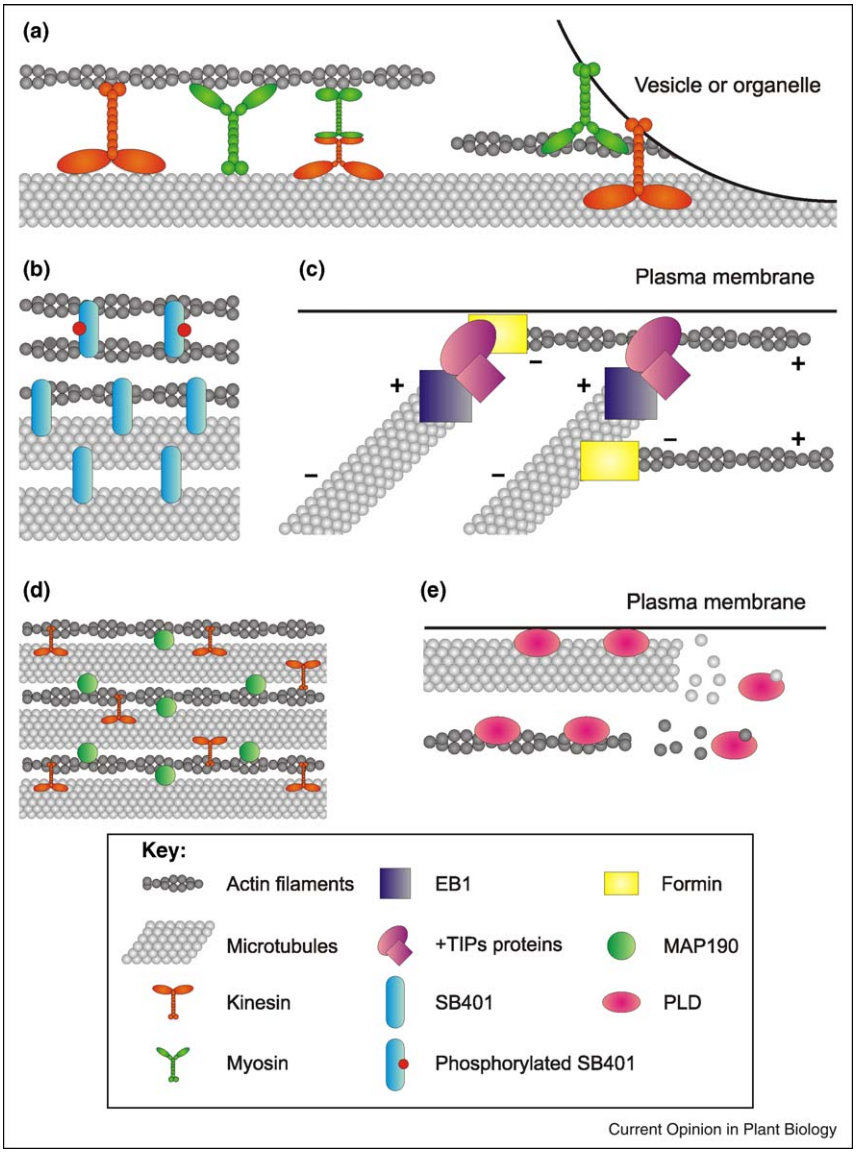
Meeting on cellular tracks: driving intracellular transport

Intracellular transport along AFs and MTs depends on the associated motor proteins, myosins for AFs and kinesins and dyneins for MTs. In higher plants, homologs of both myosins and kinesins are represented by gene families [7], whereas dynein homologs are lacking [8]. Cytoplasmic streaming and trafficking of membrane vesicles are processes generally dependent on AFs and myosin motors [9]. However, as reviewed in detail by Collings [2••], recent evidence suggests that organelle movement and positioning involves interactions of MTs with organelles like plastids and mitochondria. While the actomyosin system generates motility, MTs seem to stabilize the positioning of organelles like mitochondria [10,11•], perhaps through specific kinesins [11•,12]. The actomyosin system also assists during transverse-to-oblique (or even longitudinal) reorientation of MTs that are partly detached from the plasma membrane in fully elongated cells [13•], where MTs are passively oriented by the force generated by the actomyosin system.

On the basis of interactions described in non-plant cells, there are two possible scenarios for interactions between AFs and MTs based on the respective motor proteins. Firstly, the motor protein may interact with its corresponding cytoskeletal filament while being concurrently bound to the other cytoskeletal filament. Such interaction has been described for the MT motors kinesins binding AFs in

mammalian cells [14] and *Dictyostelium discoideum* [15] (Figure 1a). In plants, class-14 kinesins containing calponin homology domain (KCHs), which is important for binding AFs, have been identified in several species as summarized in [2••]. Among them, GhKCH1 [16] and GhKCH2 [17••] (Table 1) from cotton were shown to bind both AFs and MTs *in vitro*. These kinesins co-localize with AFs and MTs in the cortical cytoplasm as well as in the phragmoplast. Both of these kinesins may play a role in targeted deposition of material needed for directional cell expansion and

Figure 1



Schematic depiction of possible direct interactions between AFs and MTs in plants. **(a)** The interactions through motor proteins are found in all eukaryotic cells. Kinesin and myosin interact with MTs and AFs, respectively, while interacting with the other cytoskeletal filaments at the same time. Alternatively, myosin and kinesin motors interact to create an integrated motor, which uses both AFs and MTs as a transport track. Organelles or vesicles, which use both tracks for their intracellular movement, may interact with both types of motors that alternate in their function as tracks are switched. Both motors bound to one cargo can interact functionally even without a physical interaction, increasing the affinity to the track and enhancing the processivity of each other [22•]. Whereas all types of interactions were described other organisms, only kinesin-mediated interaction between MTs and AFs was identified in plants [16,17••]. **(b)** Bundling of AFs and MTs through the activity of SB401 protein may play a role in the positioning of organelles. SB401 activity is regulated by phosphorylation that decreases its affinity to MTs without influencing bundling of AFs [24]. **(c)** The interaction between MTs and AFs mediated by complexes of proteins interacting with plus-ends of MTs (+TIPs). +TIP protein EB1 interacts through other microtubule plus-end-binding proteins with actin-associated protein formin [35,37]. In addition, some proteins of +TIP family interact with AFs directly, as was shown for APC [42] or CLIP-Associated Protein CLASP [43]. Also, formin might interact through specific domains with both AFs and MTs [40,41]. **(d)** Both kinesin [17••] and MAP190 [55] may integrate the function of AFs and MTs in the phragmoplast of dividing plant cells. **(e)** Plasma membrane-associated lipid-hydrolyzing enzyme PLDδ may interact with both AFs and MTs or with actin and tubulin monomers integrating them into the downstream signaling pathways [56,58••].

Table 1

Plant proteins interacting physically with both AFs and MTs

Protein (Ref.)	Organism/cell type	Localization or association	Function
Kinesins with calponin homology domains (KCHs)			
GhKCH1 [16]	*Gossypium hirsutum* cotton fibers	Cortical MTs and transversal cortical AFs	Coordination of AFs and MTs during cell expansion
GhKCH2 [17••]	*Gossypium hirsutum* cotton fibers	Cortical AFs and MTs; midzone of the phragmoplast	Coordination of AFs and MTs during cell expansion
OsKCH1 [18••]	*Oryza sativa* coleoptiles, tobacco BY-2 cells	Cortical AFs and MTs in etiolated rice coleoptiles, radial and perinuclear AFs in tobacco BY-2 cells	Coordination of AFs and MTs during cell elongation and division
SB401 [23••,24]	*Solanum berthaultii* pollen tubes	Cortical MTs	Coordination of AFs and MTs in organelles transport in pollen tube
MAP190 [55,60]	*Nicotiana tabacum*, BY-2 cells	Mitotic spindle and phragmoplast; nucleus during interphase	Nuclear during interphase Co-localizes with spindle and phragmoplast
Formins [47,61]	*Arabidopsis thaliana*	AFs	Control of AFs assembly Potential cross-linking of AFs and MTs
EB1 [45,62]	*Arabidopsis thaliana*	Microtubule plus-end	Potential integrator of protein complex assembly on plus-end of MTs interacting with AFs
CLASP [46•]	*Arabidopsis thaliana*	Microtubule plus-end	Promotes microtubule stability Potential cross-linking of AFs and MTs
TANGLED [49••,50]	*Zea mays*, *Arabidopsis thaliana* Marks the cortical region where fusion of phragmoplast with plasma membrane occurs Distantly related to APC Potential cross-linking of AFs and MTs	MTs Deposited in a microtubule-dependent manner into the cortical region of PPB	
PLDdelta [58••]	*Arabidopsis thaliana*	MTs Tubulin and actin in pull-down assay	Initiation of signaling pathways, integration of AFs and MTs into signaling pathways

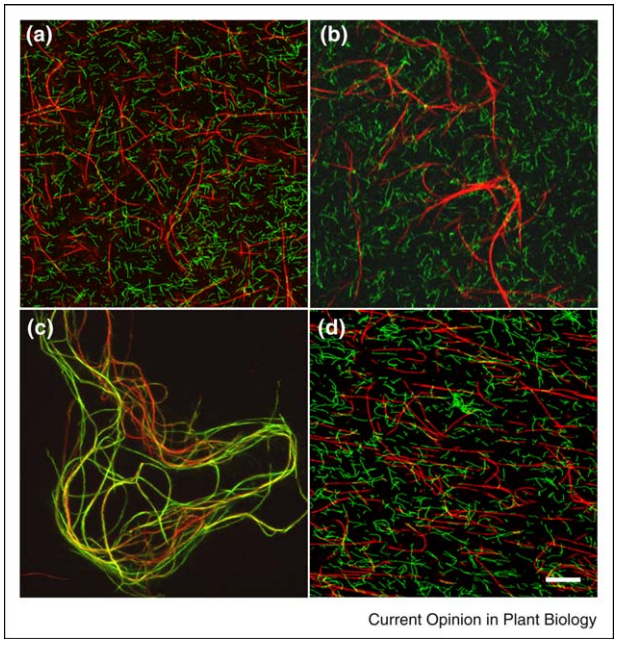
formation of new cell plate during cytokinesis. Additionally, KCHs might play a role also in cycling cells as it has been shown for recently characterized rice OsKCH1 [18]. The opposite situation, in which the actin motor myosin binds to MTs, has been described for frog oocytes [19] where the cross-linking action of myosin Myo10 is necessary for nucleus anchoring and spindle assembly (Figure 1a). However, such interaction has not yet been described for plant myosins. Secondly, two motor proteins may interact with each other either through a physical interaction as shown in yeast [20] and mice [21], or indirectly through association with the same cargo as demonstrated using an *in vitro* technique [22•] (Figure 1a). In cells where intracellular transport depends on both AFs and MTs, the quick change of transport tracks can be facilitated by the presence of two motor types on the same cargo. This may be the case of plant mitochondria that move along both AFs and MTs [10], although such physical or functional interactions between kinesins and myosins remain to be demonstrated in plants.

The coordination between AFs and MTs in transporting plant organelles may be also assisted by non-motor proteins. Recently identified microtubule-associated protein SB401 from *Solanum berthaultii* has been shown to bind and bundle both AFs and MTs *in vitro* (Figure 2) and to co-localize with cortical MTs in pollen tubes [23••] (Table 1). In addition, its bundling activity requires the formation of dimers, and is regulated by phosphorylation that decreases its affinity to MTs without influencing bundling of AFs [24] (Figure 1b). It seems that this protein may regulate the transport and positioning of organelles in pollen tubes characteristic for their highly active cytoplasmic streaming. SB401 homologs have been found exclusively in Solanaceae and there are no similar proteins known outside the plant kingdom.

Meeting in the cortical cytoplasm: regulating cell expansion and division

The coordination between AFs and MTs in the cortical cytoplasm in close vicinity of plasma membrane plays a key role during directional expansion of both tip-growing cells and diffusely growing cells as well as in the positioning of the plane of cell division. In tip-growing cells (root hairs and pollen tubes), AFs are necessary for the growth itself [25], while MTs maintain the directionality of growth [26] and determine the site of exocytosis of cell wall material, as reviewed in [27]. In diffusely growing cells, MTs provide tracks for the movements of cellulose synthases and hence directional deposition of cellulose,

Figure 2



Confocal images of *in vitro*-induced gradual bundling of MTs and AFs after the addition of SB401 protein. In the absence of SB401, taxol-stabilized rhodamine-labelled MTs and Alexa-488 phalloidin-stabilized AFs are distributed uniformly **(a)**. After the addition of SB401 MTs start to form bundles **(b)** and later also AFs form bundles that co-localize with bundles of MTs **(c)**. Importantly, addition of antibody against SB401 restores original state of MTs and AFs confirming reversible bundling role of SB401 **(d)**. Scale bar 10 μm. Reproduced with the permission from [23••].

the main factor controlling cell expansion [28], for review see [29]. However, mutations in actin and actin-associated proteins as well as pharmacological treatments with actin drugs also induce various growth defects including inhibition of cell elongation [2]. This suggests an important, but not yet fully understood role of AFs in diffuse growth. Microtubule interaction with cortical AFs may also occur in the exact positioning of the cell plate at the division site during cytokinesis, as predicted by the cytoskeletal structure preprophase band (PPB) that forms in the cortical cytoplasm before mitosis. While MTs of the PPB depolymerize before mitosis, AFs remain in the cortical cytoplasm throughout cell division [6], possibly including also an actin-depleted zone in the place of the PPB [30].

A candidate mechanism that may integrate the functions of AFs and MTs is manifested during the expansion and shaping of cells with complex shape such as leaf epidermal pavement cells and trichomes. Here, the formation of lobes is under the control of ROP (Rho of plants) GTPases and their interacting proteins, which stimulate the alignment of MTs in the neck of the lobe and polymerization of AFs inside the lobe [31]; for review see [32,33]. However, this regulatory system does not seem to include physical interaction between AFs and MTs.

Promising candidates for direct interaction between AFs and MTs in the cortical cytoplasm may be found within a family of plus-end-tracking proteins (+TIPs). +TIPs represent a class of diverse proteins associated with the growing ends of MTs (plus-ends) that include structurally unrelated, motor and non-motor proteins. At the plus-end of MTs, they often form protein complexes that control the dynamics and organization of MTs and their interactions with membranes, organelles and proteins [34]. +TIPs have been implicated in the control of AFs in the cell cortex in yeast and mammalian cells through interaction with the actin-nucleating factors, formins. In fission yeast, reorganization of AFs in the cell tip is modulated by the interaction with plus-ends of MTs, which is the location of a protein complex consisting of tea1p, tea4p, tea2p (kinesin motor), tip1p (a +TIP CLIP 170, Cytoplasmic Linker Protein-like protein) and End-Binding 1 (EB1)-like protein mal3p [35,36]. At the cell tip, tea4p directly interacts with formin for3p, which becomes activated and induces reorganization of cortical AFs [35]. In mammalian cells, two +TIPs, EB1 and adenomatous polyposis coli (APC) proteins interact with formin mDia to stabilize MTs [37]. Formins contain sequence motifs known as formin homology (FH) domains that are responsible for interactions with either monomeric (FH1) or filamentous actin (FH2) [38]. Interestingly, FH1/FH2 region of mDia was shown to bind MTs directly [39•]. Direct interaction of FH2 domain with MTs was reported also for the *Drosophila* formin Cappuccino [40] and animal formins with unusually structured FH domains [41]. Therefore, formins may form a direct link between AFs and MTs. Importantly, some proteins of the +TIP family interact with actin directly as well, as has been shown for APC [42] and CLIP-associated protein CLASP [43], making the +TIPs possible candidates for cross-linkers of AFs and MTs (Figure 1c).

Orthologs of EB1 have been found in *Arabidopsis thaliana* [44,45] and CLASP was identified as a plus-end-interacting protein as well [46•]. Similarly, formins constitute a large protein family in plants [47,48]. It has been proposed that dynamic MT plus-ends reaching the cortical cytoplasm in the root hair tip are involved in the control of AFs during tip-growth [27]. Interactions between AFs and MTs mediated by +TIPs may be involved also in other processes in plant cells. Before cell division, cortical AFs undergo specific reorganization and PPB is formed in the cell cortex. Here, protein TANGLED, which is distantly related to vertebrate APC, is deposited as a molecular 'memory' for the site of the future phragmoplast [49••,50]. EB1 is localized on plus-ends of MTs in the PPB before mitosis as well as MTs of the growing phragmoplast that reach the cell cortex at the cell division site during cytokinesis [51]. Although the basic components of protein complexes that facilitate interactions between MT plus-ends and AFs are conserved in plants, their precise involvement in such interactions remains to be elucidated.

Meeting during cell division: coordination in mitotic and cytokinetic apparatus

During mitosis and cytokinesis, arrays of AFs and MTs closely co-exist and play indispensable roles in the PPB [52] and the phragmoplast [53,54]. The incidence and possible role of AFs in the mitotic spindle remains to be elucidated [6]. The mechanism of AFs and MTs cooperation or interaction remains largely unknown and only a few proteins have been suggested to interact with both of them in these cytoskeletal structures (Figure 1d). Cotton kinesin GhKCH2 (Table 1), which has been shown to bind to both AFs and MTs, was found to localize also to the midzone of the phragmoplast in dividing root tip cells [17••]. Another candidate is microtubule-associated protein 190 (MAP190) that has been shown to co-sediment with both AFs and MTs *in vitro* using a crude protein fraction from BY-2 cells [55]. MAP190 was found to localize in the nucleus during interphase and associate with the mitotic spindle and phragmoplast during cell division. Although a direct interaction of MAP190 with AFs and MTs has not been demonstrated, and sequence motifs for known microtubule-binding and actin-binding domains have not been identified, there remains the possibility that MAP190 may be involved in integrating the functions of AFs and MTs during mitosis and cytokinesis.

Other proteins interacting with AFs and MTs

There are proteins that bind both AFs and MTs even though their specific function does not seem to be related to the cytoskeleton. Therefore, their interaction with the cytoskeleton may help to fulfill their primary role rather than cross-linking AFs and MTs. Phospholipase D (PLD), which belongs to a superfamily of signaling enzymes, associates with the plasma membrane and binds MTs [56] and this binding is important for the organization of MTs [57] (Table 1). Recently, both actin 7 and β-tubulin have been identified together with several other components of intracellular trafficking machinery in a pull-down assay using GFP-PLDδ as a bait [58••], suggesting that the isoform PLDδ may initiate important cytoskeleton remodeling processes (Figure 1e). The translational elongation factor 1α (EF-1α) was shown to bind through specific domains both AFs and MTs in animal and plant cells, as reviewed by Collings [2••], and appears to regulate cytoskeleton bundling. Bundling activity for both AFs and MTs has been also reported for plant membrane-pinching dynamin-related protein 3 (DRP3) [59].

Conclusions

Proteins connecting AFs and MTs physically are found in all eukaryotic cells, but their identification in plants is only beginning. On the basis of our current knowledge, it seems that the interaction through one bridging protein is a rather rare case in plants, as expected. However, dynamic interactions using molecular motors, signaling molecules or microtubule plus-ends appear to be well conserved in eukaryotic cells. In the next years, we expect that new proteins involved in AFs and MTs interactions will be identified using advanced techniques. More importantly, the roles of interacting proteins identified so far and their regulation will need to be elucidated.

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